Pleiotropic effects of proopiomelanocortin and VGF nerve growth factor inducible neuropeptides for the long-term regulation of energy balance

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PII: S0303-7207(20)30176-3

DOI: https://doi.org/10.1016/j.mce.2020.110876

Reference: MCE 110876

To appear in: *Molecular and Cellular Endocrinology*

Received Date: 18 December 2019

Revised Date: 14 May 2020

Accepted Date: 18 May 2020

Please cite this article as: Helfer, G., Stevenson, T.J., Pleiotropic effects of proopiomelanocortin and VGF nerve growth factor inducible neuropeptides for the long-term regulation of energy balance, *Molecular and Cellular Endocrinology* (2020), doi: https://doi.org/10.1016/j.mce.2020.110876.

Running title: Seasonal neuro-prepropeptide expression

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Disclosure: the authors have nothing to disclose.

Key words: POMC, VGF, seasonal, retinoic acid, thyroid, tanycytes

Word length: 11410

Figures: 5

Tables: 1

Abstract

Seasonal rhythms in energy balance are well documented across temperate and equatorial zones animals. The long-term regulated changes in seasonal physiology consists of a rheostatic system that is essential to successful time annual cycles in reproduction, hibernation, torpor, and migration. Most animals use the annual change in photoperiod as a reliable and robust environmental cue to entrain endogenous (i.e. circannual) rhythms. Research over the past few decades has predominantly examined the role of first order neuroendocrine peptides for the rheostatic changes in energy balance. These anorexigenic and orexigenic neuropeptides in the arcuate nucleus include neuropeptide y (Npy), agouti-related peptide (Agrp), cocaine and amphetamine related transcript (Cart) and pro-opiomelanocortin (Pomc). Recent studies also indicate that VGF nerve growth factor inducible (Vgf) in the arcuate nucleus is involved in the seasonal regulation of energy balance. *In situ* hybridization, qPCR and RNA-sequencing studies have identified that Pomc expression across fish, avian and mammalian species, is a neuroendocrine marker that reflects seasonal energetic states. Here we highlight that long-term changes in arcuate Pomc and Vgf expression is conserved across species and may provide rheostatic regulation of seasonal energy balance.

Glossary

Adrenocorticotropic hormone (ACTH)

Agouti-related peptide (Agrp)

Cocaine- and amphetamine-regulated transcript (CART)

Endorphin (END)

Melanocyte-stimulating hormone (MSH)

Neuropeptide Y (NPY)

Paraventricular nucleus (PVN)

Pro-opiomelanocortin (POMC)

Retinoic acid (RA)

VGF nerve growth factor inducible (VGF)

Ventromedial nucleus (VMN)

1. Introduction

Seasonal rhythms in energy balance are common across the animal kingdom. Some of the most striking annual phenomena that require precise control over energy stores include hibernation, torpor and migration (Jastroch et al., 2016; Davies and Deviche, 2014). The regulation of seasonal energy balance involves dynamic interactions across multiple central nervous system substrates and peripheral organs (Ebling and Barrett, 2008; Stevenson et al., 2017). The hypothalamus integrates environmental cues and internal signals required to time seasonally appropriate changes in energy balance (Mercer and Speakman, 2001; Ebling and Lewis, 2018). A series of neuropeptides localized to discrete hypothalamic nuclei are critical for homeostatic regulation of food intake and energy expenditure (Yeo and Heisler, 2012). The long-term regulation of energy balance in a predictable and regulated manner indicates that an internal rheostat provides the seasonal control of energy balance (Lewis and Ebling, 2017). This review will discuss the emerging evidence that indicates complex and dynamic interactions between the tanycytes in the hypothalamus and (an)orexigenic neuropeptides for timing seasonal variation in energy balance. We will highlight data showing that the neuropeptides, proopiomelanocortin (*Pomc*) and VGF nerve growth factor (*Vgf*) are molecular markers of long-term changes in energy balance and may reflect the actions of an internal seasonal rheostat.

2. Seasonal rhythms in energy balance

Seasonal rhythms in energy balance are regulated in part due to endogenous circannual programs that are entrained by environmental cues (Lincoln, 2019). For example, ground squirrels (*Spermophilus beecheyi*) that are maintained in a constant 12hr light, 12hr dark photoperiod display robust rhythms in body mass and torpor that approximate a seasonal cycle (Davis and Swade, 1983). In the pallid bat (*Antrozous pallidus*), the annual cycles in

body mass and food intake persistent in constant short or long photoperiods (Beasley and Zucker, 1986). These data provide compelling evidence that an internal program, or *circannual* oscillator, underlies seasonal rhythms in energy balance. However, studies designed to examine circannual control of energy balance are limited. Therefore, this review will instead, focus on the environmental regulation of seasonal changes in energy balance. The annual change in day length, also referred to as photoperiod, is the predominant environmental cue that animals use to entrain circannual programs in energy balance. In the laboratory, a simple switch in photoperiod can induce large scale changes in energy balance. The highly photoperiodic Japanese quail (*Coturnix japonica*) and Siberian hamster (*Phodopus sungorus*) are the predominant animal models used to investigate the neuroendocrine mechanisms that govern seasonal rhythms in multiple physiological systems, including energy balance (Lewis and Ebling, 2018; Stevenson et al., 2018). Due to the larger literature on the photoperiodic response of Japanese quail and Siberian hamster, our review will primarily include evidence derived from these two models. Additional data from a range of fish, avian and mammalian species are incorporated to provide a comprehensive evaluation of the neuropeptidergic regulation of seasonal energy balance.

In Japanese quail and Siberian hamsters, an increase in photoperiod from short to long days can induce a significant increase in body mass (Boon et al., 2000; Ebling and Barrett, 2008). This pattern is also observed in capture-wild birds, a simple change from short to long day lengths in the laboratory can induce vernal migration in the black-headed bunting (*Emberiza melanocephala*; Trivedi et al., 2014). The change in photoperiods induce a cascade of molecular and cellular changes that initiate body mass gain required for weight gain (Boon et al., 2000; Nakao et al., 2000) and migration (Trivedi et al., 2014). In sedentary mammalian species, the annual change in photoperiod can induce body mass loss and gain that are commensurate with the prevailing seasonal conditions in sheep (*Ovis aries*) (Lincoln

and Richardson, 1998) and most small rodents such as Siberian hamsters (Bartness and Wade, 1985; Mercer et al., 2000). Across rodents, there are a multitude of different energetic responses observed in addition to variation in body mass and include photoperiod regulation of body fat content in deer mice (*Peromyscus maniculatus*) (Nelson et al., 1992).

Short-term regulation of energy balance has focussed on the bi-directional interactions between the hypothalamus and peripheral tissues. There is a constant active regulatory process that promotes stability in the amount of body energy stored in the form of fat and the expenditure of energy stores. One of the earliest conjectures to account for food-intake regulation proposed that inhibitory signals were created that were in proportion to body fat stores and this signal(s) would stimulate brain regions to reduce the drive for food intake (Kennedy, 1953). Conversely, when body fat stores are reduced, the inhibitory signal(s) was removed and a subsequent increase in food intake drive would restore energy balance. It is now well established that the pancreatic hormone insulin is produced in plasma circulation at levels proportional to fat content and when injected into the brain reduced food intake (Woods et al., 1979). Subsequent genetic knockout studies identified leptin, a peptide hormone produced by body fat stores (i.e. adipose tissue) and could also reduce food intake when injected into the brain (Harvey, 2003). Leptin levels vary seasonally in association with fat stores in a range of highly seasonal mammals and include Siberian hamsters (Horton et al., 2000), woodchucks (Concannon et al., 2001), sheep (Marie et al., 2001) brown bats (Richardson et al., 2018) and red deer (Gaspar-Lopez et al., 2009). However, long-term rheostatic energy balance is not driven by leptin as mammals exhibit seasonal leptin resistance (Rousseau and Loudon, 2013). The role of leptin in birds is not well characterised to date. Leptin appears to have multiple effects in birds and the current evidence indicates it is not involved in regulating energy balance (Boswell and Dunn, 2015). These findings

suggest that long-term changes in energy balance are generated in the central nervous system (i.e. brain) and not mediated by peripheral signals derived from adipose stores.

3. Neuropeptides involved in the control of energy balance

The hypothalamus is critical for the integration of hormonal signals and regulation of seasonal energy balance (Mercer and Speakman, 2001; Ebling and Lewis, 2018). Experimental paradigms that employ simulated changes in day length via photoperiodic manipulation of seasonal body weight have been a valuable method to separate short- and long-term regulation of energy balance. For example, Siberian hamsters maintained in long days have elevated body weights and transfer of animals to short days induce a gradual decrease (Figure 1A). Food deprivation of hamsters in long day and short-day conditions result in a rapid decrease in body weight (Figure 1B). Acute food deprivation provides a reliable manipulation to identify neuroendocrine substrates involved in short term energy balance and appetite regulation. Conversely, seasonal changes in neuroendocrine substrates that do not exhibit short term changes in response to food deprivation can be interpreted as long-term regulators of energy balance and appetite. The arcuate nucleus is the predominant nucleus that expresses the neuropeptides involved in anorexigenic and orexigenic signalling. The four 'first order' neuropeptides include neuropeptide Y (*Npy*), agouti-related peptide (*Agrp*), Cocaine and amphetamine related transcript (*Cart*), and proopiomelanocortin (*Pomc*) (Figure 2) (Yeo and Heisler, 2012). *Npy* and *Agrp* act in the arcuate nucleus to stimulate food intake, whereas *Cart* and *Pomc* are generally associated with the reduction in food intake. Here we will discuss the role of hypothalamic neuropeptides for the regulation of seasonal energy balance.

3.1 – Neuropeptide Y

Npy is a major orexigenic neuropeptide expressed in the arcuate nucleus and stimulates food intake by acting in the paraventricular (PVN) and ventromedial nuclei (VMN) (Beck, 2006). *Npy* injections directly into the hypothalamus induce potent increases in acute (e.g. 6-8hr) food intake in multiple species including mice (Morley et al., 1987), rats (Clark et al., 1984), sheep (Miner et al., 1989) and chicken (Kuenzel et al., 1987). Food restriction studies demonstrate that acute removal of food induced *Npy* arcuate nucleus expression in rats (O'Shea and Gundlach, 1991) and sheep (McShane et al., 1993). Siberian hamsters show an acute, food restriction induced increase in *Npy* levels, but no long-term photoinduced changes in expression (Reddy et al., 2001; Rousseau et al., 2002; Bao et al., 2019). Moreover, Siberian hamsters that received intracerebroventricular injections of NPY were observed to increase food intake, but the response was similar in long- and short-day males (Boss-Williams and Bartness, 1996). In the semi-desert Jerboa (*Jaculus orientalis*) there was no significant difference in *Npy* expression between spring and autumn conditions despite large changes in body weight (Talbi et al., 2016). These data indicate that *Npy* in rodents is acutely involved in food intake but is not a major contributor to the long-term maintenance of energy balance.

In Soay rams arcuate *Npy* significantly increased in conjunction with higher voluntary food intake in sheep housed in stimulated long day photoperiods compared to short day photoperiods (Anukulkitch et al., 2007). However, other reports show that *Npy* expression does not change between long day and short-day photoperiod conditions but does show a rapid increase in food restricted animals (Archer et al., 2004). Using *in situ* hybridization, Dobbins and colleagues (2004) did not identify a change in *Npy* expression across photoperiod- or testosterone-treatments; but testosterone did increase cell size. Altogether these data indicate that seasonal variation in *Npy* expression is driven in part by testosterone and may be tied to reproductive function and not the long-term regulation of energy balance.

In ewe, the number of *Npy* cells in the arcuate nucleus are significantly higher in short day compared to long day animals (Skinner and Herbison, 1997; Clarke et al., 2000). The decrease in *Npy* during the non-breeding anestrus stage are likely oestrogen independent as ewes treated with subcutaneous implants of oestrogen did not affect *Npy* expression. In red deer (*Cervus elaphus*), there is a remarkable sex-difference in seasonal variation in *Npy* arcuate nucleus expression. During the breeding season, the male arcuate nucleus expressed more *Npy* compared to females during the non-breeding season (Barrell et al., 2016). These patterns suggest a sex-dependent regulation of seasonal appetite that may reflect a neuroendocrine switch in homeostatic energy balance.

Male song sparrows (*Melospiza melodia*) have more fat and muscle during the nonbreeding short days conditions compared to breeding long day birds. *Npy* immunoreactivity is significantly greater during the nonbreeding season (Fokidis et al., 2019). However, in the subtropical Indian weaver bird, there is no significant change in *Npy* expression across four distinct seasonal breeding states (Surbhi et al., 2015). Overall, the data indicate that *Npy* is critical for the short-term homeostatic regulation but is not required for long-term rheostatic changes in energy balance.

3.2 – Agouti-related peptide

Agrp is another orexigenic neuropeptide that shows high levels of co-expression with *Npy* cells (Sohn et al., 2013). In mouse models, ablation of *Agrp* neurons significantly reduced food intake (Gropp et al., 2005) and acute optogenetic or pharmacogenetic stimulation of *Agrp* neurons induce food intake (Aponte et al., 2011, Krashes et al., 2011). Indeed, there is ample support that *Agrp* is central for the short-term homeostatic regulation of food intake and energy balance. However, the evidence to indicate a role in long-term, seasonal change in energy balance is less convincing.

Arcuate nucleus expression does not show a significant difference between seasonal energetic states in Siberian hamsters (Rousseau et al., 2002; Bao et al., 2019) or sheep (Archer et al., 2004). Yet, male hamsters and rams that had restricted food access showed increased hypothalamic *Agrp* expression (Archer et al., 2004, Bao et al., 2019). *Agrp* overexpression using adeno-associated virus that targeted the Siberian hamster hypothalamus significantly increased food intake and a 20% gain in body mass (Jethwa et al., 2010). Remarkably, Siberian hamsters that received the *Agrp* viral vector decreased body mass when transferred to short-day lengths despite the higher levels of food intake. These data indicate that *Agrp* is strongly implicated in the acute, short term homeostatic regulation of energy balance, but does not impact the long term, rheostatic regulation of energy balance. Furthermore, *Agrp* expression in the Arctic Charr hypothalamus does not vary across the seasons even though there are large changes in body mass (e.g. approx. 100% increase) during the transition from summer to winter conditions (Striberny et al., 2015). Altogether, *Agrp* in fish and mammalian species is not involved in the long-term timing of energy balance, but instead, is integral for the short-term maintenance of energy balance.

3.3 – Cocaine and amphetamine related transcript

Late in the 1990s, cocaine and amphetamine related transcript (*Cart*) was discovered in the mammalian hypothalamus (Kirstensen et al., 1998). *Cart* was identified as an anorexigenic neuropeptide with high levels of expression in the arcuate nucleus. A reduction in circulating leptin concentration is associated with reduced *cart* expression (Xiao et al., 2005). In mice, an exogenous leptin injection produced an increased *cart* expression (Kristensen et al., 1998) and leptin receptors are expressed on CART-expressing cells (Elias et al., 2001). In male Wistar rats, the active fragment of Cart (55-102) when injected directly into either the VMN or arcuate nucleus resulted in a significant increase in feeding (Abbott et

al., 2001). However, injections of Cart (55-102) into the third ventricle of the hypothalamus inhibited feeding (Abbott et al., 2001) suggesting anatomically localized effect on food intake. Several studies have indicated that *Cart* is acutely regulated by energetic state (Yeo and Heisler, 2012) and food deprivation significantly decreased *Cart* expression (Kirstensen et al., 1998).

The evidence for a long-term role of *Cart* for the regulation of seasonal energy balance is lacking. In Siberian hamsters (Rousseau et al., 2002; Bao et al., 2019) and sheep (Archer et al., 2004), *Cart* expression was significantly decreased after food restriction, but did not exhibit seasonal variation in hypothalamic expression. In some circumstances, such as restricted exposure to short days (i.e. 14 days) or developmental photoperiodic programs, *Cart* expression can be induced in Siberian hamsters (Khorooshi et al., 2008; and Adam et al., 2000; Mercer et al., 2003). In some fish species, there is no significant seasonal variation in *Cart* expression, yet they show marked annual variation in energy balance and food intake (MacDonald and Volkoff, 2009; Striberny et al., 2015). In migratory songbirds, there was an increase in hypothalamic *Cart* expression during the onset and maintenance of the photoinduced vernal migration period (Agarwal et al., 2019).

3.4 – Proopiomelanocortin

Unlike *Npy, Agpr and Cart*, the neuropeptide *Pomc* gene includes a family of pre-prohormones that individually lead to the synthesis of melanocyte stimulating hormone (MSH), adrenocorticotropic releasing hormone (ACTH), β-lipotropin hormone and β-endorphin (Figure 3A). *Pomc* neurons are expressed in the arcuate nucleus and the nucleus of the solitary tract in the brainstem (Cone, 2005). In Siberian hamsters there is a significant increase in arcuate *Pomc* transcript levels in long day ‘obese’ animals. Studies that have used *in situ* hybridization (Reddy et al., 2001; Rousseau et al., 2002), quantitative PCR (Bao et al.,

2019) and RNA-sequencing (Bao et al., 2019) have established high *Pomc* levels in hamsters maintained in long day conditions (Table 1). In the photoperiod-sensitive F344 rat, high-fat feeding in short day and long day rats had no effect on *Pomc* expression (Ross et al. 2015) despite the known link between leptin (increased by high-fat feeding) and *Pomc* expression. Evidence to support the long-term, seasonal regulation of energy balance is provided from acute food restriction studies. In hamsters, studies that have removed food for 16-48 hours have revealed that *Pomc* expression remains constant and does not decrease as shown in mouse models (Reddy et al., 1999, Rousseau et al., 2002; Bao et al 2019).

Studies that sought to determine photoperiodic variation in *Pomc* metabolites revealed that hypothalamic MSH concentrations and immunoreactive α-MSH were greater in long day compared to short day hamsters (Logan and Weatherhead, 1980). Subsequent studies in hamsters suggested that α-MSH radioimmunoactivity and β-END immunoreactivity are significantly higher in short day Siberian hamsters (Helwig et al., 2013) and Syrian hamsters (Juss et al., 1991). In Syrian hamsters (*Mesocricetus auratus*) *Pomc* levels are greater in long day compared to short day conditions in gonadally intact animals; however, the photoperiodic difference is removed after castration (Bittman et al., 1999). Hamsters that received testosterone implants were found to have higher *Pomc* labelled cells in the arcuate nucleus, suggesting a gonadal feedback response in this species (Bittman et al., 1999). Studies that have examined the responsiveness of the melanocortin system using the MC4-R agonist MTII in Siberian hamsters showed that acclimatized to long or short days resulted in a similar inhibition of food intake despite large photoperiodic differences in body weight (Schuhler et al., 2003). These findings indicate that the melanocortin pathway provides a reliable short-term response but is not involved in the long-term regulation of energy balance. However, it remains to be determined if the endogenous receptor for β-End, the μ-opioid receptor, sensitivity and/or function varies seasonally in hamsters.

In male and female sheep, food intake is high in long day summer day lengths and then decreases when animals are transferred to short day lengths; *Pomc* expression in the arcuate nucleus is significantly increased in short day sheep (Anukulkitch et al., 2007; Archer et al., 2004). Similar to hamsters, there was no effect of food restriction on *Pomc* expression in long day male sheep, but there was a decrease in short days (Archer et al., 2004). As ovariectomized sheep retain seasonal variation in *Pomc* expression in the arcuate nucleus with highest levels during the breeding period in February, the hormonal mechanisms that drive seasonal changes in energy balance are independent of reproductive state in this species (Clarke et al., 2000). An investigation of the metabolites of *Pomc* cleavage revealed that immunoreactive levels of β-endorphin secretion in pituitary tissue were greater in short days compared to long days; there was no consistent change in β-lipotropin in rams (Ebling and Lincoln 1987); whereas in other reports, there are greater β-Endorphin levels in long- compared to short-day ewes (Skinner and Herbison, 1997). In juvenile male and female sheep there are strong seasonal changes in α-MSH hormone and β-Endorphin with a 25-fold increase in animals transferred from long to short days (Lincoln and Baker, 1995). In other ungulates such as male red deer, there are significantly higher γ-MSH in the arcuate nucleus during the non-breeding season; however, female red deer showed the opposite pattern (Barrell et al., 2016).

In Japanese quail, a period of *photoinducibility* occurs in which birds held on short days and transferred to long days exhibit a rapid increase in multiple photoperiodic genes 12h-14h after light onset (Yoshimura et al., 2003). Hypothalamic *Pomc* expression in quail significantly decreased in quail after long-term exposure to stimulatory long days and are paralleled by increased body weight (Yoshimura et al., 2003; Boon et al., 2000). Across the entire photo-induced seasonal cycle, *Pomc* expression varies with increased expression in short-day photosensitive, long day photostimulated and then decrease during the development

of long day photorefractory quail (Banerjee and Chaturvedi, 2018). Across mammalian and avian species, arcuate *Pomc* expression provides a reliable long-term marker of energy balance and is robust against short-term changes in food intake or food restriction. Altogether, these data support the conjecture that *Pomc* and metabolites are a fundamental molecular component for the rheostatic regulation of energy balance (Figure 4).

3.5 – Hypothalamic *Vgf*

A second preprohormone that has pleiotropic effects on the seasonal regulation of energy balance includes *Vgf* (Figure 3B; Jethwa and Ebling, 2008). Photoperiodic effects on VGF expression in the arcuate nucleus of Siberian hamster provided early molecular evidence that it may be involved in the neuroendocrine control of seasonal energy balance (Barrett et al. 2005; Jethwa et al., 2007; Bao et al., 2019). VGF and VGF-derived peptides are generally downregulated in short-day photoperiod in Siberian hamster in the arcuate nucleus and some other cell populations (Jethwa et al 2007, Lisci et al., 2019), however in a sub-division of the arcuate nucleus, the dorsomedial posterior arcuate nucleus, upregulation was observed in the same photoperiod. After a switch from short- to long-day photoperiods, VGF expression decreased rapidly in the dorsomedial posterior arcuate nucleus (Barrett et al 2005). Central infusion of the VGF-derived peptide TLQP-21 decreased food intake and body weight in long photoperiod (Jethwa et al 2007; Lisci et al., 2019) while systemic infusion has no effect in long photoperiod (Lisci et al 2019). Furthermore, analysis using VGF gene overexpression have shown reduction in body weight gain and energy expenditure in long photoperiod (Lewis et al 2017). Taken together, these findings unambiguously demonstrate functional involvement of VGF and VGF-derived peptides in seasonal energy balance regulation and may also form part of an internal rheostat.

4. Hormonal regulation of the neuroendocrine control of energy balance

Hormones, such as melatonin, thyroid hormone and retinoic acid are a few well-characterized chemical messengers that provide systems-wide orchestration of seasonal changes in energy balance. Here, we will briefly describe the photoperiodic regulation of these hormones in the context of the seasonal regulation of *Pomc* expression.

4.1 – Melatonin

In seasonal mammals, metabolism and feeding behavior are synchronised primarily by annual changes in day length/photoperiod, which is the most consistent environmental cue (Dawson et al., 2001; Goldman, 2001). The neuroendocrine mechanisms by which changes in photoperiod are translated in the brain to drive seasonal reproduction are well understood and have been extensively reviewed elsewhere (Dardente et al. 2019; Helfer et al. 2019). In non-mammalian vertebrates, light acts via at least three different pathways with corresponding photoreceptors located in the eyes, the pineal gland and the hypothalamus (Perez et al., 2018) whereas in mammals this is mediated by light input exclusively through the eye via the retinohypothalamic tract to the suprachiasmatic nucleus in the hypothalamus (Stevenson et al., 2017). The day length is then neurochemically translated by the nocturnal secretion of melatonin from the pineal gland. The main site of photoperiodic control by melatonin action is the pars tuberalis of the pituitary gland (Williams and Morgan 1988). Studies in a variety of seasonal species, including sheep, hamster and F344 rats have demonstrated that melatonin-responsive cells in the pars tuberalis regulate the secretion of thyrotropin stimulating hormone and this in turn regulates thyroid metabolism in the hypothalamus (Helfer et al., 2019). Melatonin secretion from the pineal gland is necessary (Kelly et al., 1994; Prendergast and Freeman, 1999; Prendergast et al., 2013; Saenz de Miera et al., 2014) for the regulation of seasonal mammalian energy balance. However, interestingly, seasonal

rhythms can be entrained in European hamster independent of melatonin signal (Monecke et al. 2013). Whether melatonin has direct effects on neuropeptides involved in energy balance is not well characterized. Indeed, melatonin receptors are expressed in the rodent arcuate nucleus (Drew et al., 2001). Melatonin receptor knockout mice show increased time feeding and reduced *Pomc* expression in the arcuate compared to wild-type mice (Fischer et al., 2017). Moreover, melatonin injections stimulated *Pomc* expression in wild-type mice indicating a strong short-term homeostatic role for the control of energy balance. Whether melatonin also provide long-term rheostatic regulation of seasonal energy balance requires further investigation.

4.2 - Thyroid hormone

Across all vertebrates, seasonal variation in hypothalamic thyroid hormone appears to be the primary driver for the neuroendocrine control of reproduction and energy balance (Dardente et al., 2014). Photoperiodic induced changes in thyrotropin stimulating hormone acts in a paracrine manner through thyrotropin stimulating hormone-receptor expressing cells located in the tanycytes along the ependymal layer of the third ventricle. The local concentrations of thyroid hormones: thyroxine and triiodothyronine are governed by deiodinase enzymes *Dio2* and *Dio3*. It has been inferred that the photoperiod-driven changes in deiodinase enzyme regulates thyroid hormone bioactivity and availability in the hypothalamus. In long photoperiod, an increased expression of *Dio2* predicts to increase triiodothyronine levels, whereas in short photoperiod *Dio2* decreases and an increase of deiodinase enzyme *Dio3* is thought to inactivate triiodothyronine. Changes in *Dio2* and/or *Dio3* expression between short and long photoperiod have been demonstrated in several seasonal species (Dardente et al., 2014), but experimental evidence for this prediction stems from studies in Japanese quails, where hypothalamic triiodothyronine levels are strongly

379 increased in long photoperiods (Nakao et al., 2008). This long photoperiod increase in
380 triiodothyronine levels has also been reported in F344 rats, although here the changes are
381 much smaller despite large changes in *Dio2* and *Dio3* expression (Ross et al. 2011). In F344
382 rats, thyrotropin-stimulating hormone not only increases *Dio2* expression, but also directly
383 inhibits *Dio3* expression (Helfer et al. 2013). Triiodothyronine implanted directly into the
384 hypothalamus could prevent short-day induced gonadal involution and body weight loss
385 (Barrett et al., 2007). Furthermore, exogenous administration of triiodothyronine to hamsters
386 housed in short day conditions result in a body weight gain and testicular recrudescence
387 (Freeman et al., 2007; Murphy et al., 2012; Henson et al., 2013; Banks et al., 2016). 2 weeks
388 of daily subcutaneous triiodothyronine injections in male Siberian hamsters housed in short
389 day conditions was observed to induce body mass gain and elevated hypothalamic *Pomc* and
390 *Cart* expression (Bao et al., 2019). While triiodothyronine availability in the hypothalamus
391 seems to be a conserved mechanism in seasonal animals, species- and daylength-specific
392 differences in photoperiod time measurement must be considered when interpreting results.
393
394 *4.3 – Downstream pathways of thyroid hormone*
395 Similar to thyroid hormone, there is ample evidence to support a role for retinoic acid
396 in the transcriptional regulation of the photoperiodic response in the hypothalamus. In F344
397 rats, hypothalamic retinoic acid concentration is under strong photoperiod regulation and
398 increased in long photoperiod relative to short photoperiod (Helfer et al. 2012). These
399 increased retinoic acid levels are accompanied by profound changes in retinoic acid transport,
400 binding proteins and synthesis enzymes in tanycytes (Shearer et al. 2010; Helfer et al. 2012;
401 Shearer et al. 2012b; Tavolaro et al. 2015). Likewise, retinoic acid transport proteins and
402 receptors are upregulated in tanycytes of Siberian hamsters housed in long photoperiod (Ross
403 et al. 2004; Barrett et al. 2006; Petri et al. 2016; Bank et al. 2017). In rats there is evidence

that retinoic acid signalling functions downstream of thyroid hormone signalling. Retinaldehyde dehydrogenase 1 (*Raldh1*) gene encoding the enzyme that catalyses the conversion of retinal to retinoic acid, which is reduced in animals exposed to short photoperiod, is upregulated by thyroid hormone (Stoney et al. 2016). Further upstream, changes in retinoic acid signalling genes are blocked by pinealectomy and increased by melatonin injections in Siberian hamsters and F344 rats, respectively (Ross et al. 2004; Helfer et al. 2012) indicating that retinoic acid signalling is a crucial part of the functional seasonal signal transducing pathway downstream of melatonin and thyroid hormone. Until recently, photoperiodic regulation of the retinoic acid signalling has only been shown in seasonal rodents; however a new report has highlighted that the retinoic acid pathway is necessary for photoperiodic response in monarch butterflies (Iiams et al. 2019). It will be important to analyse a photoperiodic response of the retinoic acid pathway in other vertebrates, but these results suggest an evolutionary conserved role for retinoic acid in seasonal biology.

While changes in hypothalamic retinoic acid pathway correlate with seasonal changes in body weight and food intake, to date no study has shown a direct link with energetic neuropeptides. A microarray analysis in F344 rats indicates that energy neuropeptides do not change after administration of retinoic acid directly into the brain (Helfer et al. 2016) and verification using *in situ* hybridization has confirmed these results for the neuropeptide NPY (Helfer and Morgan, unpublished). However, this study looked at short-term effects of retinoic acid and this is unlikely to drive sufficient long-term changes in appetite regulatory genes. Interestingly, retinoic acid increases VGF expression and promotor activity in human neuroblastoma cell lines and it has been hypothesised that low retinoic acid levels in short photoperiod can explain the reduced VGF expression (Lewis et al 2016). Given that retinoic acid signalling is induced by thyroid hormone (Stoney et al 2016), it is not surprising that

thyroid hormone implanted into the hypothalamus of hamster blocks the short photoperiod induced increase in VGF mRNA expression (Lewis et al 2016). These data provide a possible mechanistic framework for a signalling cascade driving seasonal energy balance regulation where VGF lies downstream of thyroid hormone regulated retinoic acid signalling.

5. Tanycytes as the central mechanisms for the seasonal regulation of energy balance

5.1 Tanycytes as the central seasonal pacemaker

Tanycytes are a glia cell population that line the third ventricle of the hypothalamus and extend into the arcuate nucleus and ventromedial hypothalamus. In mice, tanycytes are chemosensing and detect, and respond to circulating amino-acids and glucose (Lazutkaite et al., 2017; Benford et al., 2017; respectively). Given the hodology with adjacent nuclei implicated in food intake (i.e. arcuate) and the ability to respond to nutrient signals, tanycytes have been strongly implicated in the control of energy balance (Bolborea and Dale, 2013).

Tanycytes have been also been well-characterised as important structural, cellular and molecular pathways that contribute to seasonal regulation of energy balance (Helfer et al. 2019; Ebling and Lewis, 2018; Stevenson and Kumar, 2017). Immunolabelling for the tanycyte marker vimentin is decreased in short photoperiod, a switch to long day conditions tanycytes form long radial processes reaching into the appetite regulating centres of the hypothalamus (Kameda et al. 2003; Bolborea et al. 2011; Migaud et al. 2015; Helfer et al. 2016). Until recently it was suggested that tanycytes actively transport leptin into the hypothalamus to regulate energy balance regulation (Balland et al. 2014), however a recent study has shown that tanycytes do not express leptin receptors or respond directly to leptin therefore it is unlikely they are involved in hypothalamic leptin signalling (Yoo et al. 2019). Nevertheless, studies from seasonal F344 rats show that tanycytes express *Pomc* mRNA and, in contrast to *Pomc* mRNA expression in the arcuate nucleus, this is strongly regulated by

photoperiod (Ross et al. 2009; Ross et al. 2015). These findings indicate that tanycytes are important for the seasonal rhythms in energy balance and could be the cellular population that comprise the central node for a rheostatic mechanism (Ebling and Barrett, 2008).

5.2 Tanycytes as a neurogenic niche

Cyclical changes in newborn cells (e.g. neurogenesis) has been proposed to serve as the basis of seasonal rhythms (Hazlerigg and Lincoln, 2011). Indeed, there is ample evidence for seasonal patterns of neurogenesis in the avian and mammalian telencephalon (Sherry and MacDougall-Shackleton, 2015; Migaud et al., 2015). Tanycytes are a stem cell niche and a source of proliferating cells for appetite regulatory neurones (Lee et al. 2012; Haan et al. 2013; Robins et al. 2013). Tanycytes express several neural stem cell markers including nestin, vimentin, dopachrome tautomerase, as well as Wnt and Notch components and the hypothalamic progenitor-specific transcription factors Rax (Helfer et al., 2019; Dardente et al., 2019). Tracing studies have provided evidence that tanycyte cells can either mature into NPY, AgRP or POMC neurons (Kokoeva et al 2005, Pierce and Xu, 2010) or glia cells (e.g. astrocytes and microglia; Haan et al 2013, Hazlerigg et al 2013). Proliferative activity of tanycytes was confirmed in a majority of seasonal animals, including hamster, sheep and F344 rats and it was recently suggested that their neurogenic potential might explain variations in seasonal phenotype between photoperiodic species (Helfer et al. 2019). In Syrian hamster and sheep, bromodeoxyuridine labelling is higher under short photoperiod (Hazlerigg et al 2013, Batailler et al 2018) and likewise in F344 rats Ki57 labelling is increased (Shearer et al 2012b) suggesting high levels of cell proliferation under short photoperiod. The importance of cell proliferation/neurogenesis in the photoperiodic response is best exemplified by experiments that manipulated cell proliferation using the antimitotic drug Ara-C to prevent neural progenitor cells from dividing. In sheep, doublecortin labelling

is decreased after Ara-C administration and directly affects seasonal reproduction (Batailler et al 2018).

5.3 Epigenetic modifications and long-term regulation of energy balance

An emerging mechanism that may be involved in long-term, rheostatic regulation of the neuroendocrine control of energy balance includes rhythmic epigenetic modifications (Stevenson, 2017a; Stevenson, 2018). Seasonal variation in DNA methylation and associated *de novo* DNA methyltransferase enzymes have been observed in hibernating ground squirrels (Alvarado et al., 2015), Japanese quail (Nakao et al., 2008), redheaded buntings (Sharma et al., 2018) and Siberian hamsters (Stevenson and Prendergast, 2013; Stevenson et al., 2014; Stevenson 2017b) to name a few. DNA methylation, evidenced by an increase in *de novo* DNA methyltransferase expression and global hypothalamic DNA methylation, was upregulated by long photoperiod and inhibited by exogenous melatonin in Siberian hamsters (Stevenson et al 2013). Interestingly, photoperiod and ovarian steroids also regulate DNA methyltransferase and global DNA methylation in Siberian hamster testes and uterine tissues (Lynch et al 2016; Coyle et al., 2019) implicating DNA methylation in the control of timing seasonal changes in peripheral reproductive tissues. A microarray study in Japanese quail comparing cDNA expression in hypothalami from short and long photoperiod indicated that *Dnmt3a* is rapidly increased in response to the first long day stimulation (Nakao et al. 2008). In addition to DNA methylation, studies in Siberian hamster and F344 rats have shown expression of another epigenetic enzyme, histone deacetylase *Hdac4*, to vary in a similar magnitude and temporal pattern to *de novo* DNA methyltransferases in response to altered photoperiod (Stevenson et al 2017b; Stoney et al 2017). *Hdac4* is expressed in tanycytes and profoundly upregulated by long photoperiod. Intracerebroventricular injections of thyroid stimulating hormone induced the expression of *Hdac4* in F344 rats housed in short

photoperiod (Stoney et al 2017). In mice, offspring born to mothers that received high-fat, high-sucrose diets were observed to increase body weight up to 32-weeks of age (Zheng et al., 2015). These pups were found to have increased *Pomc* expression and reduced DNA methylation of the *Pomc* promoter (Zheng et al., 2015). Conversely, post-weaned pups fed a high fat diet gained weight and significantly increased DNA methylation in the *Pomc* promoter (Marco et al., 2013). Even though these data provide a complex picture for epigenetic regulation of energy balance, the patterns indicate that long-term regulation may, in part, be controlled by epigenetic modifications of neuropeptides implicated in food intake (i.e. *Pomc*; Benite-Ribeiro et al., 2016).

6. Conclusions

In this review, we have highlighted the long-term rheostatic regulation of *Pomc* and *Vgf* for the neuroendocrine control of seasonal energy balance. Evidence was drawn from fish, avian and mammalian species and we argued that the tanycytes are the primary cellular node that governs seasonal plasticity in arcuate *Pomc and Vgf* expression. The current data indicate that photoperiodic driven changes in hypothalamic triiodothyronine and retinoic acid may be the local signals that entrain seasonal rhythms to coordinate energetic gain and loss. It is still unclear how *Pomc* and *Vgf* regulate long-term rhythms in energy balance. It is likely that *Pomc* and *Vgf* have independent, yet convergent effects on energy balance. Intraventricular infusions of VGF-derived peptide TLQP-21 does not impact hypothalamic *Pomc* expression in Siberian hamsters (Jethwa et al., 2007). These data indicate that VGF regulates food intake and energy balance via alternative pathways that *Pomc, Agrp, Npy or Cart.* There are several avenues for future research directions that should delineate the causal roles for tanycytes, triiodothyronine/retinoic acid, and *Pomc/Vgf* expression.

First, other adipokines have emerged as important hormones in energy balance. Of these, chemerin has been studied in detail in seasonal rodents (Helfer and Wu 2018). Hypothalamic chemerin mRNA levels are strongly regulated by photoperiod with high levels in long photoperiod in photoperiod-sensitive F344 rats. Infusion of chemerin into the brain of F344 rats housed in short photoperiod mimics a long photoperiod response and triggers increased body weight/food intake (Helfer et al. 2016). Intriguingly, the increase in food intake does not correlate with the expression of *Pomc* mRNA which is downregulated in these rats.

Second, Wnt/β-Catenin pathway is well-known for its role in cell proliferation, differentiation and neurogenesis in a variety of vertebrate species (Helfer and Tups 2016). A microarray analysis in F344 rats subjected to long and short photoperiods provided initial evidence that the Wnt/β-Catenin pathway is associated with the photoperiodic response (Ross et al. 2011). Subsequent studies in F344 rats and Siberian hamsters confirmed a photoperiod-dependent expression of several components of the Wnt pathway in tanycytes and adjacent areas (Helfer et al. 2012; Boucsein et al. 2016). Interestingly, hypothalamic Wnt signalling genes are not regulated by thyroid stimulating hormone but are activated by the pars tuberalis-derived peptide hormone neuromedin U (Helfer et al. 2013) thus it remains to be investigated whether the Wnt pathway acts pleiotropically or as part of the functional photoperiodic system.

Finally, it is currently unclear if retinoic acid directly regulates (an)orexigenic genes or acts in concert with other hormones (i.e. triiodothyronine). One conjecture is that retinoic acid and triiodothyronine coordinate annual changes in hypothalamic cell proliferation, neurogenesis and/or epigenetic modifications that contribute to timing seasonal rhythms. Support for this notion is evidence that retinoic acid signalling is well known for regulating cell proliferation and differentiation (Shearer et al. 2012a). and further substantiated by the

discrete locus of the family of retinoic acid genes in tanycytes and the ability of tanycytes to proliferate. Consistent with this idea, acute injection of retinoic acid into the third ventricle of rats housed in short photoperiod increases retinoic acid responder 2 (*Rarres2*) gene expression which in turn drives changes in tanycytic morphology and plasticity (Helfer et al. 2016).

Acknowledgements

This work was funded in part by a Leverhulme Trust Research Project Grant (TJS) and the Academy of Medical Sciences, the Wellcome Trust, the Government of Business, Energy and Industrial Strategy and the British Heart Foundation and Diabetes UK [SBF004\1063] (GH). We would like to thank the British Society for Neuroendocrinology for their continuous support of our research.

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Figure legends

Figure 1 - Rheostatic and homeostatic regulation of energy balance. Photoperiodic and food restriction paradigms provide robust, reliable and repeatable manipulations to demonstrate long- and short-term mechanisms involved in energy balance, respectively. (A) Siberian hamsters maintained in long-day (LD) photoperiods maintain greater body weight. A simple decrease in photoperiod to short days (SD) (i.e. 9L:15D) induces a gradual decline in body weight and reflects the long-term rheostat. (B) Siberian hamsters in both LD and SD show a robust decrease in body weight when food restricted (FR) for 16 hrs compared to *ad libitum* (AL) conditions. It is important to note that the degree of body weight loss in LD and SD conditions is indicative of a consistent homeostatic response. Asterisks indicate significance at $P<0.005$

Figure 2 – Short-term, homeostatic neuroendocrine regulation of energy balance. The arcuate nucleus (Arc) in the hypothalamus consists of neuronal populations that co-express neuropeptide Y (Npy) and agouti-related peptide (Agrp) (indicated in red), and proopiomelanocortin (Pomc) and cocaine- and amphetamine-related transcript (Cart) (indicated in blue). Both neuronal populations project to the paraventricular nucleus (PVN) and lateral hypothalamus (LH) and impose opposite effects on food intake and appetite. Short-term homeostatic signals primarily derived from leptin and insulin signalling act on leptin receptors (LEP-R) (indicated in green) and insulin receptors (IR) (indicated in orange) in the arcuate nucleus to regulate acute changes food intake.

Figure 3 - Structure and metabolites of Proopiomelanocortin and VGF nerve growth factor inducible preprohormones. (A) *Pomc* is cleaved into multiple bioactive molecules and include melanocyte-stimulating hormones (MSH), adrenocorticotropic hormone (ACTH), corticotropin-like intermediate peptide (CLIP), endorphin (END) and Lipotropin. (B) *Vgf* also has multiple metabolites that are cleaved into NAPP129, TLQP-

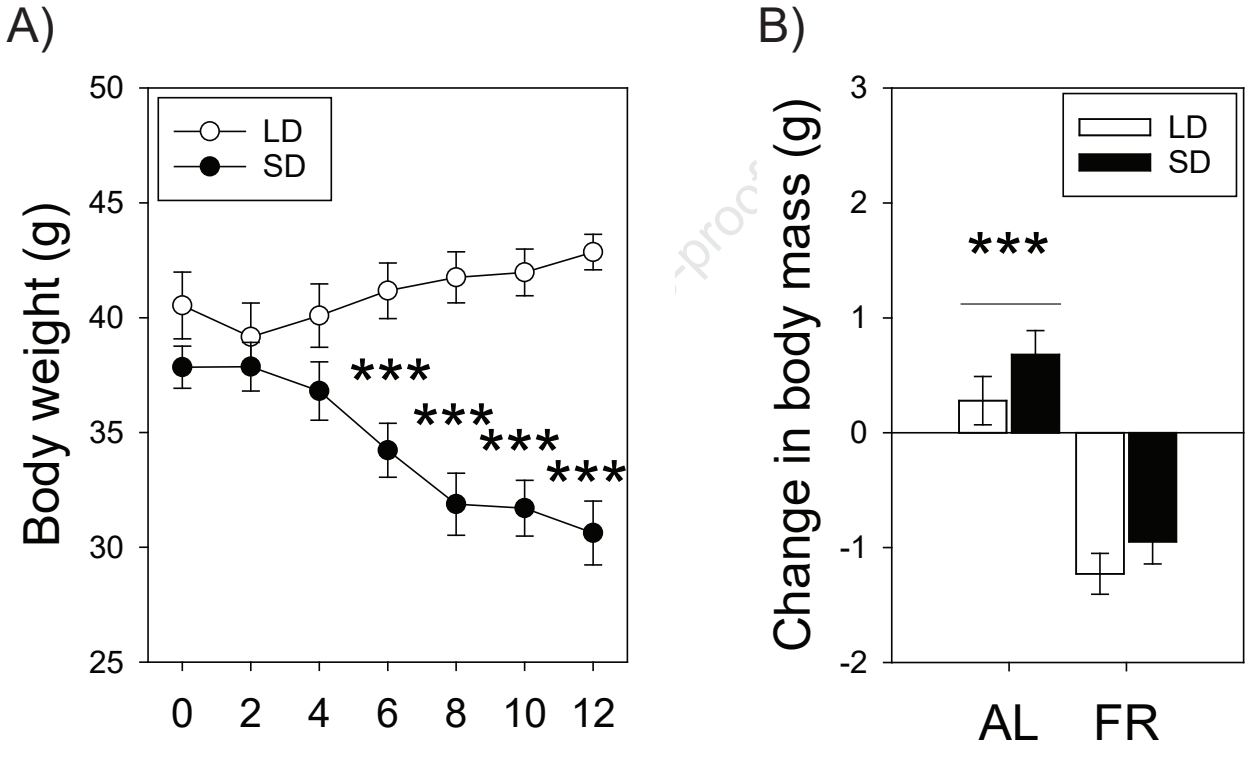
62, TLQP-21, HHPD-41, AQEE-30, LQEQ-19. Rheostatic control of *Pomc* and *Vgf* derived peptides provides long-term, pleiotropic effects to time seasonal physiological and behaviour.

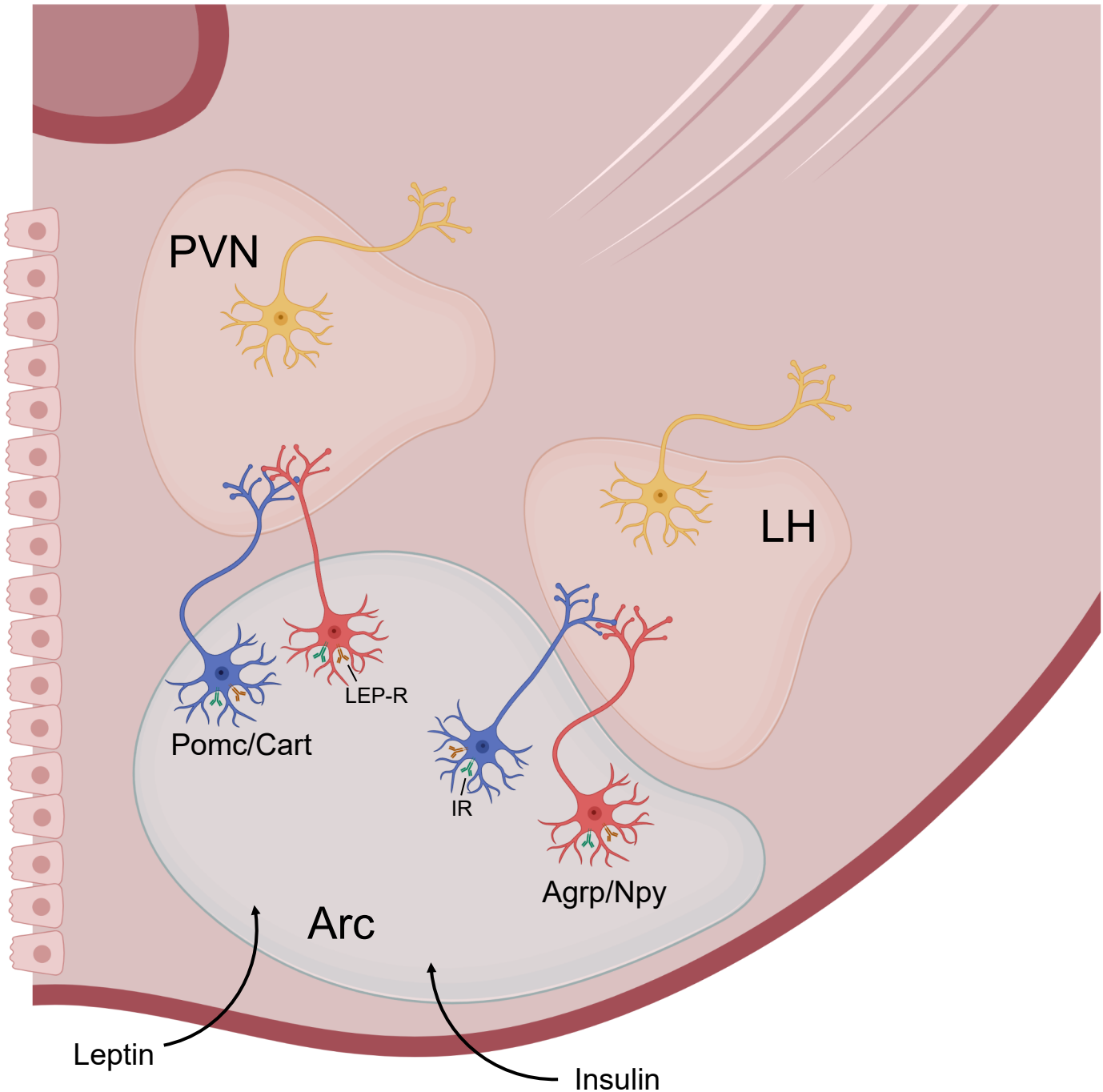
Figure 4 - Anorexigenic and orexigenic neuropeptide responses to rheostatic and homeostatic challenges. In male Siberian hamsters, hypothalamic *Pomc*, but not *Cart, Npy* nor *Agrp* expression is significantly higher in long day (LD) compared to short day (SD) photoperiods. Conversely, *Cart, Npy* and *Agrp* are upregulated in food restricted (FR) hamsters in both LD and SD conditions compared to *ad libitum* (AL) fed animals. *Pomc* expression remained consistent despite the activation of short-term homeostatic responses to food deprivation. Asterisks indicate significance at $P<0.05$.

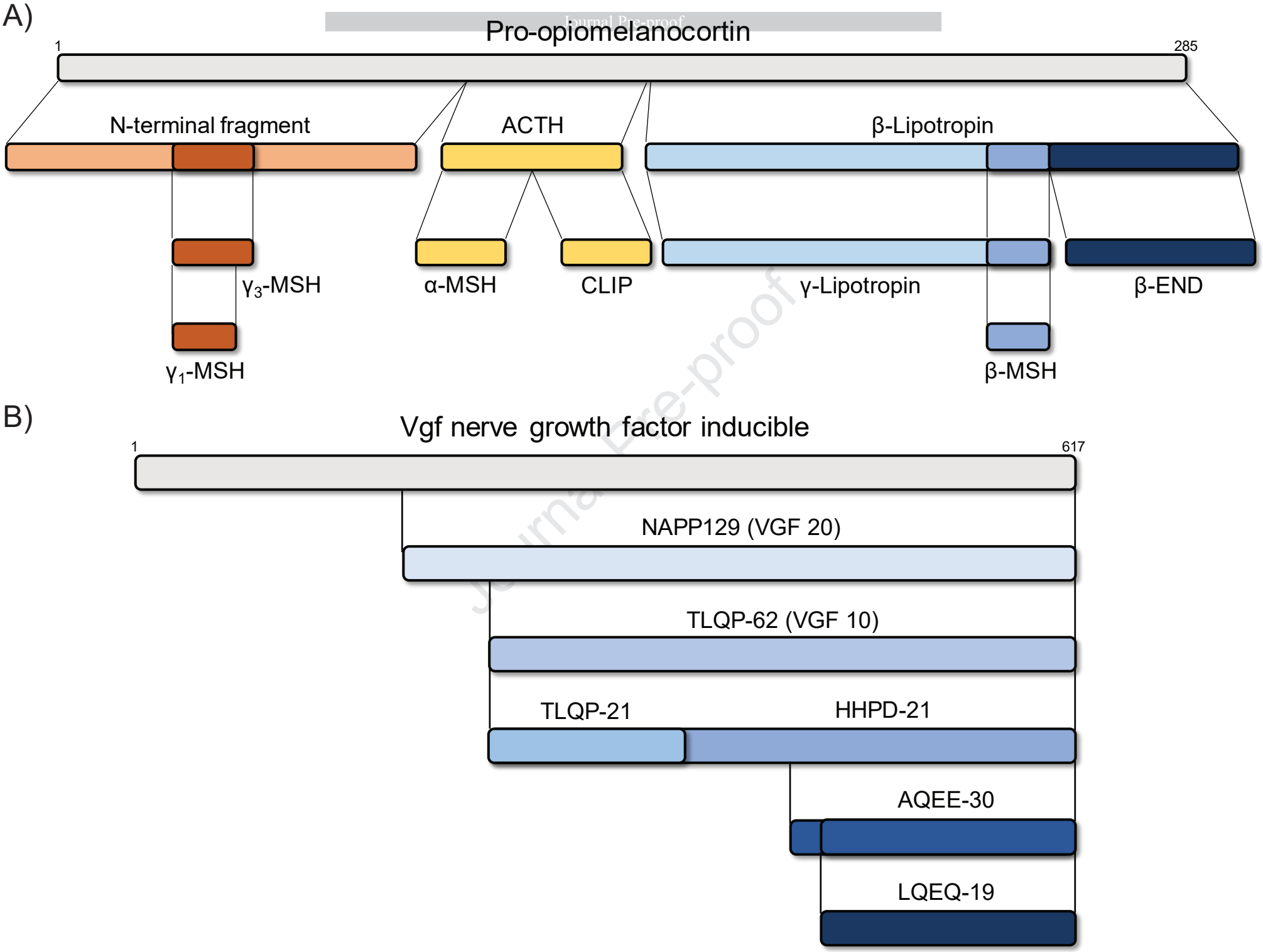
Figure 5 – Long-term rheostatic neuroendocrine regulation of energy balance. Proopiomelanocortin (Pomc) and VGF nerve growth factor inducible (Vgf) expression in the arcuate nucleus (Arc) show long-term rhythms in expression that reflect changes in energy balance. In long day breeding animals, tanycytes are retracted from the basal membrane in the median eminence and facilitate neuroendocrine-pituitary communication. Increased thyroid hormone, triiodothyronine (T3) and retinoic acid (RA) drive an increase in Pomc expression and subsequent increased body mass. Tanycyte cells in the 3rd ventricle layer are critical for the regulation of local T3 and RA concentrations and may provide neurogenic and/or epigenetic programs involved in the rheostatic control of energy balance. In short day photoperiods, there is greater tanycyte innervation (determined by vimentin expression) and increase contact with the basal membrane in the median eminence. The absence of T3/RA signalling may be involved in the photoperiodic increase in Vgf and decreased body mass.

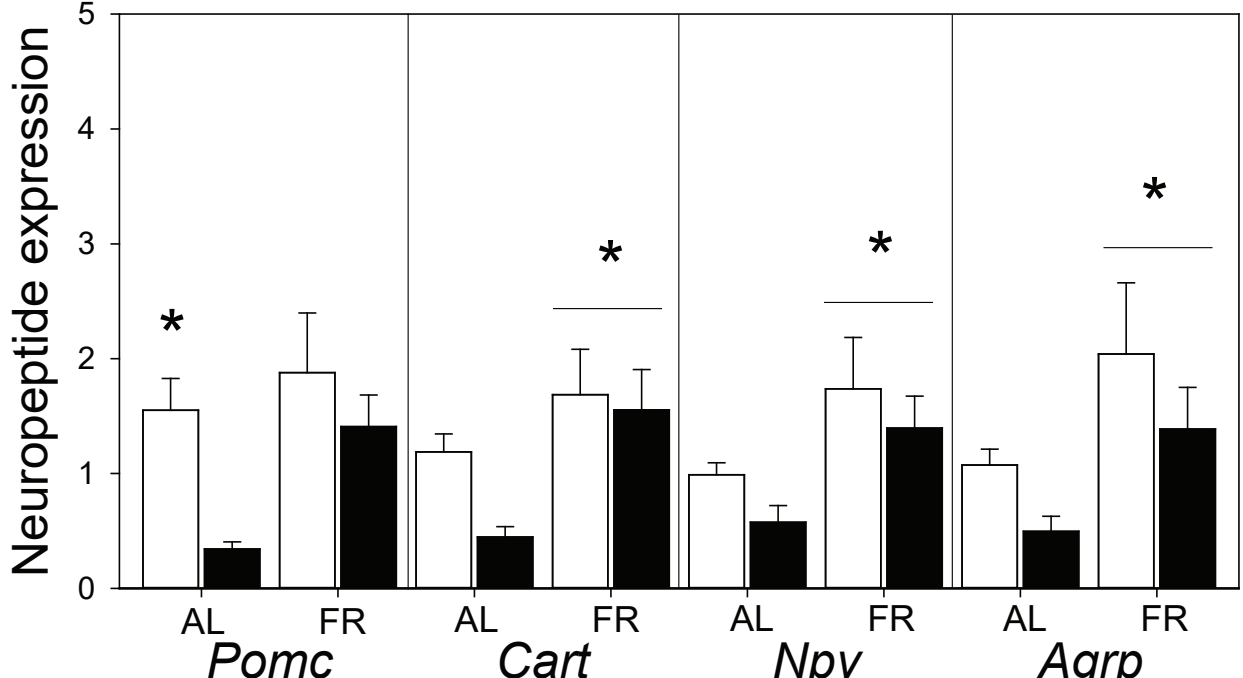
Table 1 Photoperiodic regulation of (an)orexigenic neuropeptides in Siberian hamsters

Gene	LD vs SD	Sex	Study
Pomc	↑	M	Bao et al., 2019
	↑	M+F	Helwig et al., 2006
	↑	F	Rousseau et al., 2002
	↑	M	Reddy et al., 2001
	↑	M	Mercer et al., 2000
	↑	F	Adam et al., 2000
Cart	-	M	Bao et al., 2019
	↓	F	Mercer et al., 2003
	-	F	Rousseau et al., 2002
	↓	F	Adam et al., 2000
Agrp	-	M	Bao et al., 2019
	-	F	Rousseau et al., 2002
	↓	M	Mercer et al., 2000
Npy	-	M	Bao et al., 2019
	-	F	Rousseau et al., 2002
	-	M	Reddy et al., 2001
	-	M	Mercer et al., 2000
Mc3r	↑	F	Adam et al., 2000
Cpe	↓	M+F	Helwig et al., 2013
αMsh	↓	M+F	Helwig et al., 2013
	↓	M+F	Helwig et al., 2006
βEnd	↓	M+F	Helwig et al., 2013
	↓	M+F	Helwig et al., 2006




PVN
LH
LEP-R
Pomc/Cart
IR
Agrp/Npy
Arc
Leptin
Insulin




Neuropeptide expression
5
4
3
2
1
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AL
FR
AL
FR
AL
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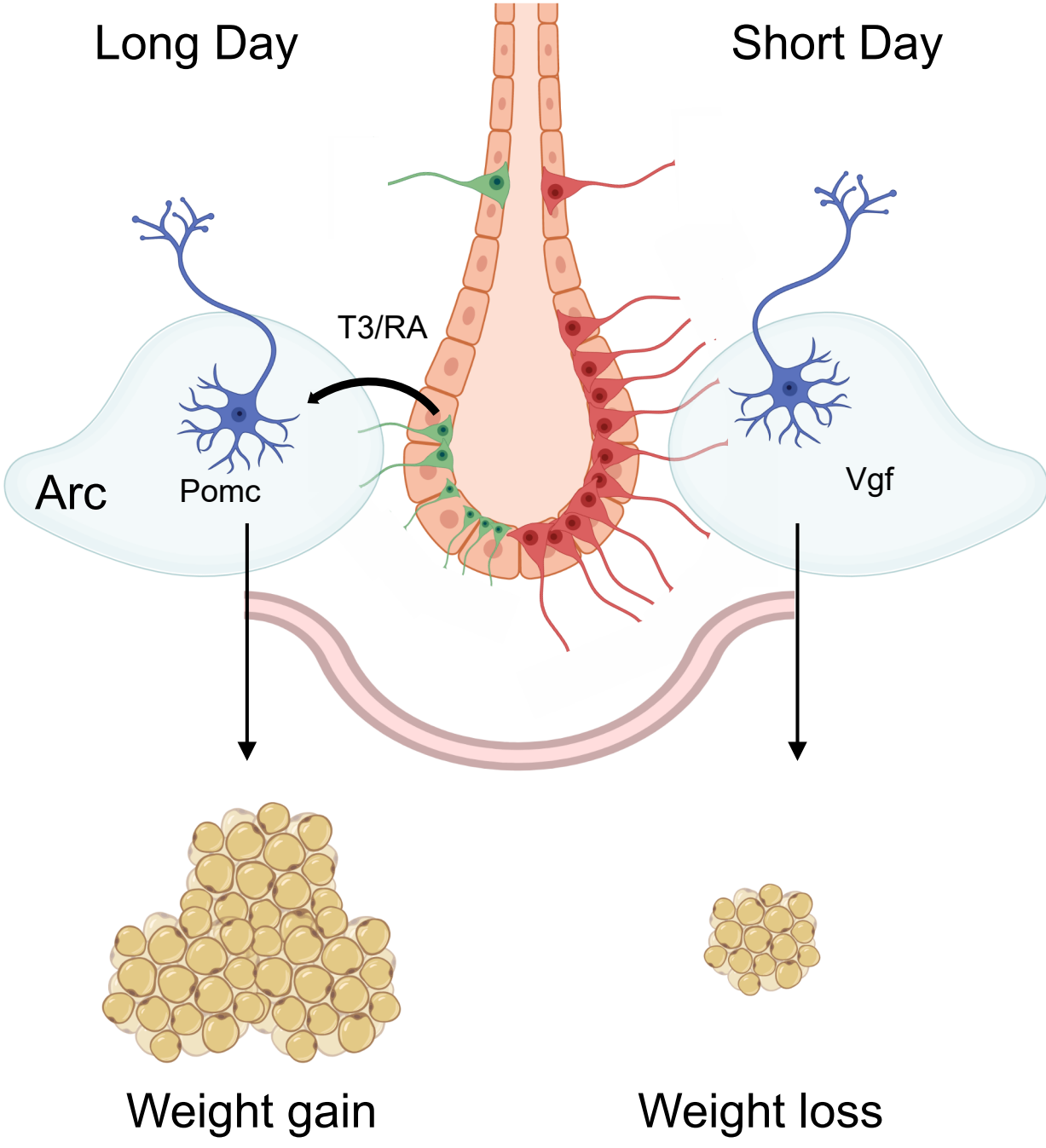
FR
Pomc
Cart
Npy
Agrp


Long Day
Short Day
T3/RA
Arc
Pomc
Vgf
Weight gain
Weight loss

Highlights

- Arcuate nucleus is a neuroendocrine substrate for long-term rhythms in energy balance
- POMC and VGF are neuroendocrine controllers of the rheostatic control of body mass
- Npy, Agrp and Cart only signal short-term homeostatic changes in body mass
- Seasonal models are essential for understanding long-term rhythms in energy balance